The expanding family of FERM proteins

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Our understanding of the FERM (4.1/ezrin/radixin/moesin) protein family has been rapidly expanding in the last few years, with the result that many new physiological functions have been abscript to these biochemically unique proteins. In the present review, we will discuss a number of new FRMD (FERM domain)-containing proteins that were initially discovered from genome sequencing but are now being established through biochemical and genetic studies to be involved both in normal cellular processes, but are also associated with a variety of human diseases.

Key words: ezrin, ezrin/radixin/moesin (ERM), 4.1/ezrin/radixin/moesin (FERM) domain, FRMD genes, Par3, Willin.

THE EXPANDING FERM FAMILY

The identification and naming of a protein from a Coomassie-Blue-stained protein gel is the starting point of an expanding number of proteins that are part of what is now called the Band 4.1 superfamily [1–3]. It is a group of proteins characterized by a conserved domain known as the FERM (4.1/ezrin/radixin/moesin) domain. Band 4.1, the prototype of the superfamily, is an erythrocyte membrane protein and a major component of the cortical cytoskeleton. The N-terminal domain of band 4.1 is highly conserved across a variety of proteins, most of which interact with both the plasma membrane and the cytoskeleton. Over time the number of known FERM-containing proteins has rapidly expanded such that, at present, approximately 50 different family members exist which have been recently categorized into three general groups comprising: (i) talins and kindlins, (ii) ERMs (ezrin/radixin/moesin), GEFs (guanine-nucleotide-exchange factors), kinases and phosphatases, and (iii) myosins and KRITS (Krev interaction trapped proteins) [4].

Within the second category are ezrin, radixin, moesin and merlin, whose functions have been reported and summarized in many excellent reviews [5–9]. In general, the ERM family together with merlin have pivotal roles in cell signalling events, including those involved in both organization of the cytoskeleton and cell proliferation. The maintenance of the sub-membrane cytoskeleton is achieved by linking actin filaments, expressed in the cell cortex, with the plasma membrane and with plasma-membrane-associated proteins. These proteins control the organization of complex membrane domains by a strict regulation of their structure and function [10]. Despite sharing similar regulatory patterns, interplay with common partners and similar subcellular localization, ERM proteins do display different tissue-specific expression patterns: ezrin is mainly expressed in polarized epithelial and mesothelial cells [11,12], moesin in endothelial and lymphoid cells [13], and radixin in hepatocytes [1,14]. This suggests incomplete functional redundancy and highlights important tissue- and context-dependent functions [6].

With regard to the ERM-related protein merlin, it is encoded by the NF2 (neurofibromin 2) gene, located on chromosome 22 [15,16], and has been demonstrated to function as a tumour suppressor gene in mice and humans. Mutations in the NF2 gene have been well characterized in neurofibromatosis type 2 disease, which also confers a predisposition to the appearance of schwannomas and meningiomas [17,18]. However, more recently the other ERM proteins have now been associated with cancer. For example, ezrin was found to be up-regulated and mislocalized to the cytoplasm and away from the apical membrane, whereas its expression in these scenarios correlated with invasiveness, cell migration and metastasis in breast cancer cell lines as well as primary breast carcinomas [19–23]. Finally, strong cytoplasmic ezrin was found to be independently associated with poorer patient survival in SCCs (squamous cell carcinomas) [24]. In a follow-up study, 131 histologically confirmed primary HNSCCs (head and neck SCCs) were prospectively analysed for cancer progression and survival. Immunohistochemical analysis of ezrin, moesin and merlin expression in tissue microarray samples of HNSCCs revealed a significant association of increased cytoplasmic ezrin with poor cancer survival [25], suggesting that ezrin is an important biomarker for HNSCC.

THE FRMD FAMILY

Initially due to the sequencing of the human genome, but now from both GWAS (genome-wide analysis studies) and proteomic studies, a crop of new FERM-domain-containing proteins have been identified in the ERM, GEF, kinase and phosphatase subset of the FERM family [4]. These have been labelled as...
Figure 1 Schematic representation of FRMD/FRMPD proteins

Protein names and gene identifiers (gi) are listed. Protein domains are annotated according to the corresponding amino acids. This representation was based on information obtained from the CDART (Conserved Domain Architecture Retrieval Tool). A colour key of the domains is provided. aa, amino acids; FA, FERM adjacent domain; FERM C, FERM C-terminal domain; FERM M, FERM middle domain; FERM N, FERM N-terminal domain; KIND, kinase non-catalytic C-lobe domain; PDZ, PSD95/Dlg/ZO1-homologous peptide-binding domain.

either FRMD (FERM domain)- and/or FRMPD (FERM and PDZ domain)-containing proteins. For some of these proteins (FRMD1 and FRMD8), they remain as hypothetical proteins with no experimental investigation; however, recently for some of these proteins there have been a flurry of publications that have highlighted their importance, either associating them with a range of different diseases or uncovering previously unrecognized associations between signal transduction pathways. These new FERM proteins include: FRMD2/FRMPD1, FRMPD2, FRMD3, FRMD4A, FRMD4B, FRMD5, FRMD6 and FRMD7 (Figure 1). A number of different isoforms have been predicted for these proteins, but for the purposes of the present review we have focused on those human isoforms where there are experimental data confirming their existence. In all cases, both biochemical and genetic studies have shown FRMD/FRMPD genes to be involved both in normal cellular processes and also with a variety of human diseases. Interestingly, where a functional role is known, the proteins are thought to act as scaffolding proteins.

BIOCHEMICAL STRUCTURE

Figure 1 shows the broad predicted structures of these proteins, indicating that all of these proteins have predicted FERM domains. The FERM domain is a cysteine-rich hydrophobic protein module of approximately 300 amino acids in length. Producing crystal structures of FERM proteins has proven to be problematic and as such only partial structures of FERM-containing proteins have been developed for ezrin [26], moesin [27,28] and radixin [29,30]. However, despite these limitations, structural studies of the FERM domain have revealed three distinct subdomains termed F1, F2 and F3 [27], which interact together to form a globular clover-leaf-shaped structure [28,31]. F1 has a ubiquitin-like structure containing a Ras-binding domain; F2 has an acyl-CoA-binding protein-like structure; and F3 contains a PTB (phosphotyrosine-binding), a PH (pleckstrin homology) and an EVH1 [Ena/VASP (vasodilator-stimulated phosphoprotein) homology 1] domain [28,31].

With regard to the FRMD family, their sequences indicate that they all have a predicted N-terminal FERM domain (Figure 1), but as yet there is no physical structure for any of these proteins. However, an unbiased fold recognition server 3D-PSSM reveals that a three-dimensional model of the FERM domain of FRMD6/Willin (14–322 amino acids) is structurally very similar to the known three-dimensional crystal structure of the moesin FERM domain (PDB code 1E5W), and so the FERM domain of FRMD6/Willin appears to contain the three previously identified subdomains: F1, F2 and F3 (Figure 2). This indicates that, despite a low sequence identity (up to 25 % for moesin) between the FERM domains of FRMD6/Willin and the ERM protein family, the structural homology between these domains is high.

As biochemical motifs, FERM domains are slightly unusual since they are able to bind a diverse range of molecules such as phosphoinositols [32], glycoporins [33], hyaluronate receptors CD44 and CD43 [10,34–37], ICAM (intercellular adhesion molecule)-1, -2 and -3 [38], actin [37,39], neurofascin [40] and the C-terminal domain of FERM-containing proteins [41]. The interplay between phospholipids and proteins is thought to help control their activation and has been described previously [42–45]. At present, FRMPD2 and FRMD6/Willin are the only cases where there is any direct evidence that the FERM domain of the FRMD proteins binds the same phospholipids as the ERM, but how these may control the binding of other proteins to the FERM domains is unknown. However, in the following sections we will summarize the known biochemical events that these proteins have now been linked to and the range of different signalling events.

FRMD2 (FRMPD1)

FRMD2 (official name FRMPD1) was first reported as a protein of 169 kDa (GenBank® accession number XP_233002) encoded by
an mRNA of 4912 nt, which contains one PDZ (Gln67–Thr135) and one FERM (Leu177–Phe401) domain, and no other readily identifiable domains. It was identified during the search of binding partners of AGS3 (activator of G-protein signalling 3) using a yeast two-hybrid screen [46]. Rat FRMPD1 exhibits 89 and 74% amino acid sequence identity with mouse (GenBank® accession number NP_001074641) and human (GenBank® accession number NP_055722) FRMPD1 respectively. AGS3 contains a GPR (G-protein regulatory) motif which binds to the Gβγ subunit of the GPCR (G-protein-coupled receptor) and stabilizes it to allow binding of the Gαi subunit to form a complete receptor complex [46]. The AGS3–FRMPD1 interaction occurs at the C-terminal domain of FRMPD1, and so the PDZ and FERM domains are not required for the interaction with AGS3. However, the distribution of GFP (green fluorescent protein)-tagged FRMPD1 in the cell cortex or membrane structures did require the presence of the PDZ and FERM domains [46]. These data suggest that FRMPD1 plays an important role in positioning AGS3 at the plasma membrane, where AGS3 can then dissociate from FRMPD1 to allow the AGS3–Gαi interaction to take place, an event that is likely to be brought about by confocalmational change of AGS3. Although there are no reported disease associations thus far, FRMD2 alteration could have multiple detrimental effects due to its impact upon normal GPCR signalling.

**FRMPD2**

*FRMPD2* (PDZK4) is a FRMD2-related gene that was initially identified as a gene that is specifically expressed in adult brain and up-regulated in synovial sarcomas [47]. FRMPD2 is a potential scaffolding protein containing an N-terminal KIND (kinase non-catalytic C-lobe domain), a FERM domain and three PDZ domains [48]. Alternative splicing yields three full-length cDNA sequences of Adult1, Fetal1/SS and Adult2/Fetal2 variants of 4241, 3980 and 3653 nt, encoding 673, 769 and 660 amino acid proteins respectively. Functional studies have concentrated on the Fetal1/SS transcript which was localized to expression just under the plasma membrane in cells which had been engineered with ectopic expression, and subsequently promoted proliferation in both T98G and COS7 cells. Conversely, reducing FRMPD2 expression using siRNAs (small interfering RNAs) in synovial sarcoma cell lines inhibited their growth [47].

Interestingly, FRMPD2 was reported to be expressed in many cell lines of epithelial origin, selectively localized at the basolateral membrane in polarized epithelial cells and thought to be involved in the formation of TJs (tight junctions) in MDCK (Madin–Darby canine kidney) and Caco-2 cells [48]. Apical-basal polarity divides the cell into two complementary membrane domains, an apical domain and a basolateral domain, which are separated from each other by cell–cell junctions (TJs and AJs (adherens junctions)). The separation between these domains is achieved by the antagonistic action of the polarity complexes, groups of proteins that define the apical and basal regions and determine the position of the junctions. The apical domain is characterized by two main polarity complexes: the aPKC [atypical PKC (protein kinase C)] complex and the Crb (Crumbs) complex [49]. Data from experiments [48] altering levels of E-cadherin (epithelial cadherin) suggested that FRMPD2 localization at the basolateral domain, at TJs and at cell–cell contacts is dependent on the presence of E-cadherin. Disengagement of E-cadherin by lowering the calcium concentration in the culture medium led to redistribution of FRMPD2 to the entire plasma membrane. This effect was reversible, supporting an E-cadherin-dependent localization of FRMPD2. Furthermore, redistribution of FRMPD2 with strong accumulation at cell–cell junctions was demonstrated after transient transfection of E-cadherin in HeLa cells, whereas specific down-regulation of endogenous E-cadherin led to loss of accumulation of endogenous FRMPD2 at cell–cell contacts [48].

The FERM domain of FRMPD2 binds specifically to PtdIns(3,4)P₂ and to a lesser extent also to phosphatidylinositol monophosphates, whereas no binding was detected to PtdIns(4,5)P₂ or PtdIns(3,4,5)P₃ [48]. PtdIns(3,4,5)P₃ is a known regulator of basolateral epithelial polarity [50,51] and, in a normal cell, PtdIns(3,4,5)P₃ is dephosphorylated at the D-5 position phosphate from the inositol ring by the inositol polyphosphate 5-phosphatases forming PtdIns(3,4)P₂ [52,53]. The interplay between PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ fits with the observed FRMPD2 expression at the basolateral domain.

Basolateral restriction of FRMPD2 also requires its second PDZ (PDZ2) domain, which showed an interaction with δ-catenin and p0071. In line with these findings, a point mutation in PDZ2, which interferes with target binding, abolished basolateral restriction of full-length FRMPD2. Taken together, these data led the authors to propose a model in which the membrane-binding activity of the FERM domain of FRMPD2, together with the binding capacity of the PDZ2 domain to p0071, determines E-cadherin-dependent basolateral localization of FRMPD2 [48]. To date, there are no further reported disease associations for FRMPD2 subsequent to the initial finding of up-regulation in synovial sarcomas [47].

**FRMD3 (4.1O)**

*FRMD3* (4.1O) was first identified from a large-scale sequencing analysis for novel human 4.1 genes as a transcript that is 2312 bp in length and encodes a protein of 553 amino acids [54]. The protein contains a FERM domain located at residues 6–181 of the protein sequence, which shares homology with mouse protein 4.1B (identity 38%, similarity 55%). The expression pattern of the human *FRMD3* (4.1O) gene in 16 adult tissues showed a transcript detected only in the ovary, whereas in the remaining 15 tissues, specific bands of the transcript could not be detected. However, in eight human fetal tissues, the specific bands of the transcript could be detected in skeletal muscle, with lower levels detected in thymus and brain. The *FRMD3* gene consists of 14 exons and 13 introns and was mapped to chromosome 9q21-9q22 by bioinformatics analysis [54]. Expression has subsequently been observed in human adult lung [55].

Data from functional assays have started to ascribe a role for this protein, but extensive biochemical analysis is lacking at present. Ectopic expression of FRMD3 promoted a reduction in clonogenicity in HEK (human embryonic kidney)-293, HeLa and A549 cells, which correlated with increased apoptosis in these cells. Furthermore, the clonogenicity of FRMD3-overexpressing HEK293 cells was restored in the presence of a caspase inhibitor, indicating that active caspases are at least in part involved in the apoptosis mediated by FRMD3 in these cells [55]. Although FRMD3 expression was found in normal lung tissue, FRMD3 expression was silenced in 54 out of 58 independent primary NSCLC (non-small-cell lung cancer) specimens compared with patient-matched normal lung tissue [55], implicating FRMD3 as a tumour suppressor gene in lung cancer.

GWAS have also implicated a number of *FRMD* genes with disease. Studies searching for diabetes loci have implicated a possible link with FRMD3 and this disease. One study assessed 360,000 SNPs (single nucleotide polymorphisms) in 820 case subjects and 885 control subjects with Type 1 diabetes. The strongest association was found at the 5’ end of the *FRMD3*.
gene, suggesting a link between FRMD3 and diabetes, although this association failed to reach genome-wide significance. Other analyses provided mixed evidence for a link, with one study in African-Americans showing an association with a diabetes risk locus rather than a direct association, whereas studies in other populations found no association with diabetes [56–59].

**FRMD4A**

FRMD4A was first identified as a binding partner of the Arf6 (ADP-ribosylation factor 6) GEF Cytohesin-1 using a yeast two-hybrid assay [60]. FRMD4A encodes a protein of 1031 amino acids containing an N-terminal FERM domain, a coiled-coil domain and a band 4.1-like domain. Amino acids 343–405 corresponding to the coiled-coil domain of FRMD4A are responsible for the interaction with Cytohesin-1. Further confirming this interaction, ectopic GFP-tagged FRMD4A was found to co-localize with Cytohesin-1 at primordial AJs and TJs in EpiH4 cells. The same study also showed that Par3 binds to FRMD4A within amino acids 565–920 in the band 4.1-like domain, suggesting that FRMD4A acts as a scaffolding protein linking Par3 to Cytohesin-1 [60]. This is of note since the Par3–Par6–aPKC–Cdc42 complex regulates the conversion of primordial AJs into belt-like AJs and the formation of linear actin cables during epithelial polarization [61]. When primordial AJs are formed, Par3 recruits FRMD4A, thereby connecting Par3 and Cytohesin-1. The authors proposed a model suggesting that the Par3–FRMD4A–Cytohesin-1 complex ensures accurate formation of Cytohesin-1 at primordial AJs into belt-like AJs and the formation of linear actin cables during epithelial polarization [61].

The FERM domain of FRMD4A shows high sequence similarity to the Grp-1 signalling partner (FRMD4B/GRSP1). This protein also localizes with and binds to Par3. Double knockdown of FRMD4A and GRSP1 in EpiH4 cells affected the formation of belt-like AJs and TJs. As expected, the localization of Cytohesin-1 at primordial AJs disappeared from the cell–cell boundaries between double-knockdown cells, supporting the idea that FRMD4A and FRMD4B/GRSP1 co-operatively recruit Cytohesin-1 to primordial AJs [60].

A GWHA (genome-wide haplotype association) study was performed in the EAD1 study cases [n = 2025 AD (Alzheimer’s disease) cases and 5328 controls] which implicated FRMD4A as a new genetic risk factor in AD [62]. The strongest association was observed at 10p13, this haplotype region appeared to be fully included within the FRMD4A gene and the association was consistently replicated in all of the data sets analysed [62].

Increased FRMD4A expression was also observed in primary HNSCCs, where high expression levels correlated with an increased risk of relapse [63]. In addition, laboratory experiments showed that FRMD4A silencing decreased growth and metastasis of human SCC xenografts in skin and tongue, reduced SCC proliferation and intercellular adhesion, and stimulated caspase 3 activity and expression of terminal differentiation markers. Interestingly, FRMD4A attenuation caused nuclear accumulation of YAP (Yes-associated protein), suggesting a potential role for FRMD4A in Hippo signalling (see below). Treatment with an HSP90 (heat-shock protein 90) inhibitor or ligation of CD44 with hyaluronan caused nuclear depletion of FRMD4A, nuclear accumulation of YAP and reduced SCC growth and metastasis [63].

**FRMD4B (GRSP1)**

FRMD4B/GRSP1 was first identified in a screen for proteins that interact with GRP1 (guanine-nucleotide-releasing protein 1), an Arf exchange factor [64]. FRMD4B/GRSP1 cDNA was isolated from a lung library and encoded a protein with a predicted molecular mass of 115 kDa. Interestingly, an extended cDNA clone from brain was also isolated, but the sequence indicated the presence of an alternatively spliced variant in brain [64]. A subsequent analysis by RT (reverse transcription)–PCR suggested that FRMD4B/GRSP1 is expressed at high levels in other tissues as well, including kidney, spleen, heart and bone marrow [65]. FRMD4B/GRSP1 contains a FERM domain, two coiled-coil domains and a serine/threonine-rich domain and interacts via its first coiled-coil domain with the N-terminal coiled-coil domain of endogenous GRP1 in intact cells. Along with GRP1, it translocates from the cytoplasm to the plasma membrane of cells stimulated with insulin [64]. Localization of GRP1 family proteins to the plasma membrane and subsequent activation of Arfs have been implicated in a variety of cellular processes, including adhesion, endocytic trafficking, cell motility, T-cell signalling, helper T-cell activation and insulin signalling [64]. Both FRMD4B/GRSP1 and GRP1 co-localize with F-actin (filamentous actin) in membrane ruffles and cortical actin-rich regions in CHO-T cells that have been stimulated with insulin or EGF (epidermal growth factor) [64], suggesting a role in F-actin function under these conditions.

FRMD4B/GRSP1 readily forms heterodimers with both GRP1 and Cytohesin-1, but not other structurally related proteins such as ARNO [66], suggesting that it acts as a scaffolding protein to recruit a multiprotein complex. As described above, FRMD4B/GRSP1 co-operates with FRMD4A to associate with Par3 [60].

In a case-control study of advanced heart failure, an analysis of approximately 30000 SNPs in 2000 candidate genes identified a common variant located in a 3′ intronic region of the FRMD4B gene that showed replicated associations in caucasian patients with advanced heart failure [67]. A subsequent re-sequencing approach confirmed a modest association with this intronic sequence variant with ischaemic heart failure [68].

**FRMD5**

FRMD5 was first identified as a gene whose transcription is down-regulated by the binding of a mutant form of p53 (p53R273H) to sequences within the first intron of FRMD5 [69]. FRMD5 was subsequently listed as a gene potentially down-regulated in side population cells from A549 cells compared with non-side population cells [70], although this differential expression was not confirmed by further analysis. FRMD5 encodes a 570 amino acid protein that shares 51% sequence similarity with FRMD3 and contains a N-terminal FERM domain [71]. The identification of any biological functions of FRMD5 has been lacking, but a recent study has provided some biochemical characterization [71]. Interestingly, GFP-tagged FRMD5 was found to localize at points of cell–cell contact in HaCaT cells. Furthermore, FRMD5 co-localized with AJ markers, such as α, β, γ and p120-catenin, but not tight junction markers, suggesting that FRMD5 is localized in the AJ [71]. Co-immunoprecipitation studies in H1299 cells showed that FRMD5 did not associate with actin, but was associated with p120-catenin via the C-terminal domain of FRMD5, whereas E-cadherin was also present in this complex. Reduced expression of FRMD5 in H1299 cells caused a decrease in E-cadherin expression but an increase in vimentin. Moreover, mesenchymal markers fibronectin and snail were up-regulated in cells where FRMD5 was knocked down, suggesting that FRMD5 is involved in the EMT (epithelial–mesenchymal transition) process [71]. Since EMT is an important mechanism of
tumour progression, both cell migration and invasion assays were performed. Data showed that knock down of FRMD5 accelerated the velocity of migration, suggesting that FRMD5 inhibits the lateral migration of tumour cells. Invasion properties of the cells in which FRMD5 was knocked down were also assessed in vitro and also by xenografts in mice. Data showed that the cells in which FRMD5 was knocked down showed decreased in vitro invasion and decreased tumour growth in xenograft culture [71]. Collectively, these data suggest that FRMD5 plays an inhibitory role during tumour progression. Both FRMD5 and FRMD3 may have wider tumour suppressor roles in other organs and cell types yet to be studied.

**FRMD6/Willin**

FRMD6/Willin was first identified by a yeast two-hybrid screen of a rat sciatic nerve library using the neuronal transmembrane protein neurofascin as bait [72]. Neurofascin is an essential receptor for the development of the mammalian nervous system, including the formation of myelination and specifically the formation of the node of Ranvier [73,74]. From this yeast two-hybrid screen, the C-terminal domain of neurofascin was found to bind to a number of different proteins: SAP102 [75], ezrin [40] and an unidentified new open reading frame encoding a novel FERM domain, which was named Willin [72]. As indicated below, Willin was subsequently reported as the human homologue of the Drosophila protein Ex (Expanded) and was given the HUGO nomenclature gene name FRMD6 [76].

FRMD6/Willin is a 614 amino acid protein with a predicted molecular mass of 71 kDa. It has high sequence similarity to merlin and ERM proteins only within the highly conserved N-terminal FERM domain, specifically between residues 14 and 322 [72]. Indeed, the FERM domain of FRMD6/Willin shares 43% protein homology with the FERM domain of ezrin, 47% with that of radixin, 45% with moesin and 46% with merlin (Figures 1 and 2). FRMD6/Willin is widely expressed in both neuronal and non-neuronal tissues, for example gene card database evaluation (http://www.genecards.org/cgi-bin/carddisp.pl?gene=FRMD6) indicates potential expression in the brain, heart, lung, liver and prostate, whereas more direct analysis of tissue has indicated expression in peripheral nerves and epithelial layers, including skin [24,72]. Furthermore, cDNA clones of Willin have been reported in human placenta, uterus and cervix [72].

The intracellular distribution of FRMD6/Willin is dependent on the cellular context. Histological staining for FRMD6/Willin on normal human oral mucosa and in SCC indicated that it is localized in the cytoplasm and in some cases at the plasma membrane [24,72]. Interestingly, FRMD6/Willin protein was also present in the nucleus of almost all UADT-SCC (SCCs of the upper aerodigestive tract) and this presence was exclusive for this protein and not members of the ERM family [24]. Differential intracellular distribution is common in ERM proteins in response to the cellular environment via conformational changes in their structure. In fact, when cultured cells reach confluency, ERM proteins tend to locate at the plasma membrane performing roles in contact inhibition and adhesion [77]. More recently, they too have been observed in the nucleus of cells, although their functions here are poorly understood [77,78].

In regions of cell–cell contact, FRMD6/Willin expression levels at the plasma membrane are increased [24,72,79] and this increase is augmented by the addition of growth factors [72]: specifically in PC12 cells, under the influence of both EGF and NGF (nerve growth factor), FRMD6/Willin translocates from the cytoplasm to the plasma membrane in a manner independent of PI3K (phosphoinositide 3-kinase) activity [72]. At present, the mechanism behind this redistribution is unknown, although studies with other FERM proteins may provide clues to a possible mechanism. In general, extracellular cues promote ERM protein activation via regulation of protein conformation changes. For example, after PtdIns(4,5)₂ binding to ERM N-terminal domains [34], ERM proteins located in the cytoplasm are activated by phosphorylation of a conserved C-terminal threonine residue by a range of different kinases: ROCK (Rho kinase) [80,81], PKCζ [82], PKCε [83], PKCθ [84], MRCK (myotonic dystrophy kinase-related Cdc42-binding kinase) [85], SLIK (Sterile20-like kinase) [86,87] and LOK (lymphocyte-oriented kinase) [88]. This phosphorylation prevents self-association between the N- and the C-termini allowing the ERM proteins to interact with diverse binding partners [6]. Upon activation, ERM proteins can translocate to the plasma membrane where their FERM domain binds to membrane-associated proteins [6]. In addition the C-terminal 34 amino acids of the ERM proteins can bind F-actin and the bridge between the actin cytoskeleton and the cell cortex is thus accomplished [31,89].

Ewing to the close homology between ERM proteins and FRMD6/Willin within the FERM domain it could be hypothesized that, in PC12 cells, FRMD6/Willin is in an inactive state and the presence of growth factors such as EGF or NGF induces FRMD6/Willin phosphorylation. Consequently, FRMD6/Willin becomes ‘activated’ and redistributes to the plasma membrane where it interacts with its binding partners. Notably, EGF is able to induce ezrin phosphorylation and to activate the Rho GTPase family [90].

With regard to the subcellular control of FRMD6/Willin distribution, there are many unknowns, but tantalizing clues are emerging. For example, FRMD6/Willin has been reported to bind phospholipids [72] and there is a number of predicted phosphorylation sites in FRMD6/Willin (http://www.phosphosite.org). In addition, FRMD6/Willin has also been shown to co-localize with actin. However, unlike the other ERM and merlin, there is no actin-binding motif within FRMD6/Willin. Moreover, the actin cytoskeleton is not necessary for FRMD6/Willin to localize at the plasma membrane, since cytochalasin-D-induced actin disruption did not alter FRMD6/Willin expression at the plasma membrane [72]. Also of note is that FRMD6/Willin protein redistribution stimulated by EGF or NGF was not observed in the other cell lines analysed [72], highlighting the importance of cellular context. However, recent data shows that FRMD6/Willin localizes with ezrin at the plasma membrane and co-immunoprecipitates with ezrin, but surprisingly not directly, with merlin [79,90a].

FRMD6/Willin is the nearest human sequence homologue to the Drosophila protein Ex. In *Drosophila*, the lack of Ex expression has been shown to be associated with overgrowth of certain structures such as the wing and imaginal discs [91], reflecting a direct role in controlling cell growth in these tissues. In addition, the *Drosophila* homologue of NF2/merlin encodes Mer (Merlin). Ex and Mer co-localize in both the cytoplasm and plasma membrane, and this interaction is accomplished through the FERM domain of Ex and the C-terminal domain of Mer [92]. Notably, these proteins differentially regulate multiple downstream pathways: Ex regulates cell cycle exit, whereas Mer regulates apoptosis [93]. The Hippo/Salvador/Warts (Hippo) pathway defines a kinase signalling cascade which regulates cell contact inhibition, cell growth, organ size, proliferation, apoptosis and cancer development by inhibiting the nuclear translocation of the transcriptional co-factor Yki (Yorkie)/YAP [94]. Hamaratoglu et al. [76] elegantly described the tumour suppressor roles of Mer and Ex and positioned these proteins as members of the Hippo

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pathway, acting upstream of the protein core kinase cassette. Two vertebrate homologues of Ex, Ex1 and Ex2, were also reported and of note was that the DNA sequence of human Ex1 is identical with FRMD6/Willin [72,76]. Thus Willin is the closest human sequence homologue of Ex, sharing 60% homology with the Ex FERM domain. While FRMD6/Willin shares similar subcellular localization with Ex, it was not able to compensate for the loss of Ex in Drosophila [79]. The C-terminal region of Ex showed no similarity to FRMD6/Willin, and indeed Ex is reported to be 1429 amino acids long, as compared with 614 amino acids for FRMD6/Willin [72,76]. The C-terminal region of Ex possesses three PPXY motifs that are essential for its interaction with the WW domains of Yki [95]. Badouel et al. [95] demonstrated that Ex and Yki localize at the apical junctions of cells in the eye imaginal disc and that Yki is a binding partner of Ex.

Interestingly, another protein called AMOT (angiomotin) has three (L/P)PPXY motifs (two PPXY and one LPXY), which bind to the WW domains found in YAP (Figure 3) [96–99]. Both structurally and in terms of sequence similarity, this resembles the binding of Yki to Ex. However, whether the LPXY motif, as well as the two PPXY motifs, is essential for the AMOT–YAP interaction is yet to be determined. Additionally, AMOTL (AMOT-like) 1 and 2 proteins, closely related to AMOT, also exist. The presence of both the LPXY and PPXY motifs has been shown to be essential for the interaction between AMOTL1 and YAP [100]. Silencing of AMOT resulted in higher mRNA levels of CTGF (connective tissue growth factor) and increased CTGF promoter activity, while the overexpression of AMOT caused a reduction in the protein levels of CTGF. However, this was not observed during the silencing of AMOTL1 [99,101]. Therefore it is possible that the role of Ex has been split over evolutionary time into the combined actions of FRMD6/Willin and AMOT [79,102], although at present there is no evidence of a direct interaction or co-operation between these two proteins. To determine whether this hypothesis is a valid one, we propose their co-expression followed by the use of functional assays. For example, the creation of a Willin–AMOT fusion protein would be useful to establish whether the chimerae protein could rescue the Ex mutant phenotype in Drosophila.

Additionally, PTPN14 (protein tyrosine phosphatase, non-receptor type 14), which also has an N-terminal FERM domain as well as two PPXY motifs, has been demonstrated to directly interact with YAP via the WW domains of YAP and the PPXY domains of PTPN14 [103,104]. The PTPN14 protein level is elevated by an increase in cell density. Expression of PTPN14 leads to sequestration of YAP in the cytoplasm, in a phosphatase-independent manner, and inhibits YAP transcriptional co-activator function, potentially through both direct and indirect effects on YAP. Conversely, knockdown of PTPN14 induces YAP nuclear retention and increases YAP-dependent cell migration [103,104]. However, the effect of PTPN14 expression on the levels of pMST1/2 [macrophage-stimulating 1 (hepatocyte growth factor-like)] and pLATS1/2 [large tumour suppressor, homologue 1/2 (Drosophila)] was not tested. Further studies on KIBRA [kidney and brain expressed protein; or WWC1 (WW domain-containing protein 1)] and AMOT, two potential PTPN14 interactors, may provide important information on how YAP localization is regulated by PTPN14 [103,104].

Components of the Hippo pathway core kinase cassette are all conserved in mammals: MST1/2 (Hpo orthologues), WW45/Sav (Sav orthologue), LATS1/2 (Wts orthologues), MOB1 (Mats orthologue) and YAP (Yki orthologue) [105]. However, some mechanistic differences occur upstream of the core kinase cassette between Drosophila and mammals [105,106]. In Drosophila, Ex and Mer form a tripartite complex with Kirb that regulates the Hippo pathway [49,107,108], but it is unclear at present if the FRMD6/Willin, Merlin, KIBRA multiprotein complex is conserved in mammals [49]. Indeed, recent data would argue against this possibility and instead suggests that KIBRA signals in an MST-independent manner directly to LATS1/2 [109,110].
The expanding family of FERM proteins

The FERM domain of FRMD6/Willin was shown to be sufficient to activate the Hippo pathway via MST1/2 and to antagonize YAP-induced phenotypes in mammalian cells [79]. FRMD6/Willin activated the Hippo pathway, inducing the phosphorylation of MST1/2, LATS1 and YAP in MCF10A and HEK (human embryonic kidney)-293T cells. Knockdown of FRMD6/Willin mimicked YAP overexpression with respect to inducing an EMT phenotype in MCF10A cells, and it decreased phosphorylation of MST1/2, LATS1 and YAP [79]. Surprisingly, in contrast with these findings, it has been reported that FRMD6/Willin acts independently of the Hippo pathway in MDA-MB-231 cells overexpressing FRMD6/Willin, with no observed phosphorylation of the core kinase cassette [111]. High cell confluency would have a significant effect on basal phosphorylation levels of the Hippo pathway components, and it is not clear what level of confluency the cells were at when tested or the methodology used in the paper [111], therefore such high basal levels may have masked any increase. Unfortunately, Hippo pathway phosphorylation in MDA-MB-231 cells upon knock down of FRMD6/Willin was not addressed. Interestingly, the same report described an inverse relationship of taxol sensitivity and FRMD6/Willin expression, where increased levels of FRMD6/Willin increased the sensitivity of MDA-MB-231 cells to taxol [111].

Events in the Hippo pathway upstream of FRMD6/Willin are slowly being elucidated. Recently, FRMD6/Willin has been shown also to physically interact with Par3 and co-operatively regulate epithelial apical constriction through aPKC-mediated ROCK phosphorylation [112]. FRMD6/Willin co-localized with Par3 in MDCK cells and co-immunoprecipitated with Par3 and aPKC in MDCK and EpH4 cells. The FERM domain and a region of the C-terminal domain adjacent to the FERM domain [termed JFR (juxta-FERM domain region)] of FRMD6/Willin was sufficient to recruit aPKC to AJs. Surprisingly, in FRMD6/Willin–GFP-expressing MDCK cells, aPKC remained at the AJs even when Par3 was depleted, suggesting that FRMD6/Willin recruits aPKC to the AJs independently of Par3 [112]. These data are important since they link FRMD6/Willin to components of the cell polarity machinery, as described above for FRMPD2 and FRMD4A. Finally, it has been shown that the association of the JFR of FRMD6/Willin with AJs is dependent on the nectin 1–afadin complex in L fibroblast cells [113].

A second connection with the polarity machinery exists: the FERM domain of Ex is recruited to the plasma membrane by its interaction with Crb [114–116]. Loss of Crb or mutations within its FBM (FERM-binding motif) results in mislocalization of Ex to the basolateral region [114–116]. An unbalanced expression of Crb results in the loss of Ex expression which then leads to the concomitant disruption of the associated Hippo pathway complexes [95,108]. Interestingly, changes in the intracellular distribution of Mer or the transmembrane protein Fat [117–120] were not observed in Crb mutant cells [116]. It is interesting to speculate that the FERM domain FRMD6/Willin may also associate with mammalian Crb proteins.

Intriguingly GWAS have linked FRMD6/Willin to both asthma and AD. One SNP in the FRMD6/Willin gene (rs3751464) has provided evidence for an association of FRMD6/Willin with asthma [OR (odds ratio) = 1.43 (1.18–1.75); P = 3 × 10−4], even with correction for multiple testing [121]. Additionally, the expression level of the FRMD6/Willin gene was consistently lower in the lungs of mice with allergic airway inflammation and it was significantly lower in human asthmatics compared with controls [121]. From a meta-analysis of AD, four intronic/promoter variants of the FRMD6/Willin gene achieved strong study-wide significance [122]. FRMD6/Willin had been highlighted in two previous GWAS, and in each case they were
related to brain function. The first was a study of hippocampal atrophy [123] and the second study used genotyping linked to three-dimensional images of structures throughout the brain [124]. Although there are currently no reports of FRMD6/Willin inactivation in cancer, FRMD6/Willin is antagonized by ezrin in its ability to phosphorylate MST1/2 [79] and so, in a wider context, ezrin may counteract the effects of FRMD6/Willin on the Hippo pathway and so act as an oncogene. The FRMD6/Willin gene is located on chromosome 14, open reading frame 31 (GenBank® accession number BC020521) of the human genome [72]. The 14q region has been described as being involved in several pathological conditions such as renal carcinoma [125], gastric cancer [126], uterine leiomyoma [127], meningiomas [128,129], gliomas [130,131], neuroblastomas [132] and gastrointestinal stromal tumours [133,134]. Loss of heterozygosity and mutations on 14q have been associated with chromosomal abnormalities on chromosome 22q [135,136], which is where the merlin gene NF2 is located. This suggests that FRMD6/Willin, like merlin, might act as a tumour suppressor.

FRMD7

Idiopathic congenital nystagmus is characterized by involuntary, periodic and predominantly horizontal oscillations of both eyes. Visual function can be significantly reduced owing to constant eye movement. A study identified 22 mutations in FRMD7 in 26 families with X-linked idiopathic congenital nystagmus [137]. All mutations identified in FRMD7 co-segregated with disease in the linked families and were absent from 300 male control chromosomes. Many mutations predicted truncated proteins or defects in exon splicing predicted to result in transcript degradation. In addition, the restricted expression of FRMD7 in human embryonic brain and developing neural retina correlated with tissues affected in this disease [137]. Thus FRMD7 is required for healthy visual function.

CONCLUSIONS AND FUTURE DIRECTIONS

FERM domains are found in a wide variety of proteins, but in particular they constitute the major, and in some cases the only, recognized functional domain present in the FRMDs (Table 1). The general three-dimensional structure of the FERM domain is consistent with its proposed role as a scaffold for numerous protein–protein and protein–phospholipid interactions. Although only partial crystal structures have been determined for the FERM domains of ezrin, radixin and moesin, computer-aided molecular modelling of other FERM domains shows that high structural homology exists, and three-dimensional configurations are maintained in the FERM domains of FRMDs despite rather low primary sequence homology. FERM domains are unusual biochemical motifs in that they are able to bind a diverse range of molecules. Interestingly, FRMD6/Willin and FRMD2/PtPi have been shown to bind phospholipids. FRMD2 was shown to bind PtdIns(3,4)P₂, whereas FRMD6/Willin was shown to bind PtdIns3P, PtdIns4P and PtdIns5P. It is possible that other FRMDs may also bind phospholipids that would mediate their activities, and future studies should explore this possibility.

A number of FRMDs interact with proteins located at points of cell–cell contact (AJs and TJs). FRMD2 (with E-cadherin/δ-catenin/p0071), FRMD5 (with p120-catenin), FRMD4A and FRMD4B (with Cytohesin-1/Par3) and FRMD6/Willin (with Par3/Par6/aPKC) are all associated with proteins located at points of cell–cell contact (Figure 4). Since these associated proteins have roles in maintaining apical and basolateral membrane domains in polarized cells, then these FRMDs may have functional roles in polarity and it will be interesting to see whether there are overlapping roles for these proteins in this process. Additionally, FRMD2/FRMPD1 has been linked to AGS3/GPCRs and FRMD6/Willin may also be involved downstream of GPCRs via the Hippo pathway. GPCRs integrate a number of diverse extracellular signals, so cross-talk with these receptors would affect many cellular signalling networks.

GWAS are beginning to link the activities of FRMDs to diverse disease settings, including diabetes, cancer and AD. Future studies should elucidate both the biochemistry and the pathophysiology of these interesting proteins in order to link their functional roles to defects in disease.

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Table 1 Summary of FRMD/FRMPD proteins described in the present review

<table>
<thead>
<tr>
<th>Protein</th>
<th>Interactions</th>
<th>Domain</th>
<th>Function</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRMD2/FRMPD1</td>
<td>AGS3</td>
<td>PDZ</td>
<td>Unknown</td>
<td>Currently no association</td>
</tr>
<tr>
<td>FRMD2</td>
<td>PtdIns(3,4)P₂, δ-catenin, p0071</td>
<td>FERM, KIND</td>
<td>Binding to PtdIns(3,4)P₂</td>
<td>Up-regulation in synovial sarcomas</td>
</tr>
<tr>
<td>FRMD3</td>
<td>Unknown</td>
<td>FERM, Three PDZ</td>
<td>Second PDZ interaction with δ-catenin and p0071</td>
<td>Down-regulated in NSCLC, linked to diabetes via GWAS</td>
</tr>
<tr>
<td>FRMD4A</td>
<td>Par3, Cytohesin-1</td>
<td>Amino acids 343–405</td>
<td>Binds to cytohesin-1 at AJ</td>
<td>GWAS linked to AD, up-regulated in HNSCC</td>
</tr>
<tr>
<td>FRMD4B</td>
<td>Par3, Cytohesin-1, GRP1</td>
<td>Amino acids 955–920</td>
<td>Binds to Par3</td>
<td>Linked to heart Failure</td>
</tr>
<tr>
<td>FRMD5</td>
<td>p120-catenin</td>
<td>FERM, Amino acids 542–732</td>
<td>Binds to Par3</td>
<td>In vitro studies linked to invasion</td>
</tr>
<tr>
<td>FRMD6</td>
<td>Phospholipids, Par3, aPKC, ezrin</td>
<td>FERM</td>
<td>Activates the Hippo pathway</td>
<td>GWAS links to AD and asthma</td>
</tr>
<tr>
<td>FRMD7</td>
<td>Unknown</td>
<td>FERM, FA</td>
<td>Unknown</td>
<td>Linked to idiopathic congenital nystagmus</td>
</tr>
</tbody>
</table>

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